CHAPTER II

METHODS AND MATERIALS

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1. Methods of Venom Extraction

The technique of catching the snakes is very simple. A wooden handle measuring about 1 mitre with a hook like an 'L' on the inferior end is used to hold the snakes. The inferior end of the handle then presses the head of the snake on the floor. Next, the head of the snake is seized, the posterior part is held firmly between the forefinger and the thumb of the left hand; the othe fingers hold the neck against the palm of the hand (Fig. 1). The mouth of the snake is opened and its poisonous fangs are placed on the inner edge of a fixed glass (Fig. 2). The venom glands now pressed upon by two fingers, the venom is extracted into the glass pot.

2. Methods of Drying Venom

After extraction, the venom is dried in a vacoum desiccator over fuse calcium chloride. The dried venom is kept in a sterile sealed tube in a cool dark place for maintenance of its potency for a long time.

3. (i) Preparation of Perfused Toad's Heart

Toads of average weight of 100 g were chosen for perfusion technique. Before carrying out the experiment, the toads were pithed and heart was exposed from the thoracic cavity. From a reservoir the Ringer's solution, pH 7.4 (perfusion fluid), was allowed to flow through a Syme's canula, from which the fluid was passed by means of a small canula, tied to the hepatic vein, to the heart and passed out by the aortae which were cut. A thread was attached to th apex of the heart by a hook and the beats were recorded at a suitable pressure

Fig I

Fig 2

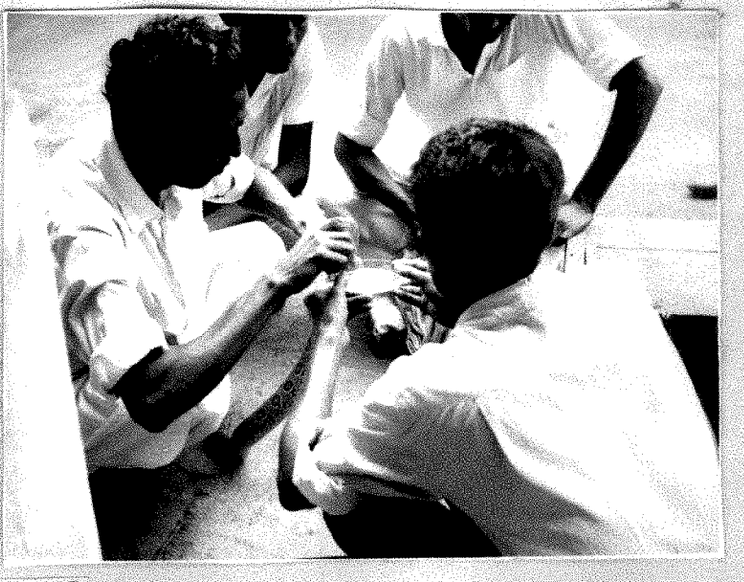

Fig 3

Figs. 1, 2 and 3

Method of extraction of venom.

The venom was extracted in the Department of Physiology, Calcutta University.

Fig. 1 shows the way by which the jaws are caught and then pressed for venom collection.

Figs. 2 and 3 - The venom is being collected in a petri dish.

by a lever provided with a sharp writing point which scratches its movement on a revolving smoked drum.

3. (ii) Preparation of Ringer's Solution

For preparation of Ringer's solution the following reagents were used:

13% NaCl	... 50 ml
2.4% $CaCl_2$	... 5 ml
2.8% KCl	... 5 ml

The volume was made upto 1 litre with water and the pH was adjusted to 7.4.

4. Technique of Measuring of Blood Pressure and Respiration

Cats of both sexes, weighing from 2 to 2.5 kg body weight, were used for the experiments. All the cats were healthy and well-adapted to the laboratory conditions.

Anesthesis

Nembutal has been used as non-volatile anesthetic and ether as the volatile anesthetic. Nembutal was given at a dose of 50 mg/kg body weight intraperitoneally.

In all cases primary induction with ether was done by putting the animal in a closed chamber in which whiffs of ether were circulated from a bottle containing ether by a pumping arrangement. The chamber was so designed that the different stages of anesthesia could be viewed through the transparent glass lining chamber. When the animal was completely anesthetized it was brought out of the chamber and placed on the kymograph table in supine position and the limbs were tied.

A tracheotomy was rapidly performed following the technique of Lidell and Sherington (1929). One limb of a glass T-tube was introduced through the slit on the trachea and secured in position. The animal was then anesthetized

with nembutal. Inhalation of ether was stopped when the administration of nembutal was completed.

Technique of Blood Pressure Recording

The blood pressure was recorded from either of the common carotid arteries. The right or left common carotid was first isolated and then tied with a cotton thread below the region of its bifurcation. Blood flow through the artery was obliterated by means of a vascular clip.

During this time the mercury manometer with its floating stylus was kept in order and checked before cannulation. The arterial canula with its rubber tube and the mercury manometer were filled with sodium citrate (4%) solution in water and care was taken so that no air bubble lingered within the circuit. A V-shaped incision was given in between the two ligatures, the canula was introduced within the artery through the incision and then tied with a piece of thread. An extra pressure, just a little greater than the blood pressure of the animal, was given within the canula, only to prevent the excess blood coming out into the canula and also into the rubber tubes. The second ligature (towards the heart) was then removed and blood rushed into the canula, pressed forward the citrate solution and thus also the column. The pressure was recorded on a smoked paper by the floating stylus.

5. Technique of Electrocardiographic Study

For this experiment six male white rabbits (1.5 ± 0.5 kg body weight) were housed in cage and kept on laboratory condition and basal diet for 7-10 days.

Now the electrocardiographic changes were noted with the help of an 8-channel electroencephalograph (Grass Instrument Co, U.S.A., Model IIID). The standard limb leads were used to pick up the electrical activity of heart.

The electrocardiographic tracing paper was allowed to move at a speed of 60 mm/sec and the calibration of the machine was 1 mv = 10 mm = 1 cm in general. Electrocardiographic records were interpreted with the principles and techniques of Graybiel and White (1946).

6. Determination of Toxicity of Venom Solution (Cobra and Russell's Viper)

In toxicology, it is, however, important to establish the smallest amount of venom just sufficient to cause death and it is designated as the minimum lethal dose (MLD) and expressed by a weight unit of this animal. The figures for minimum lethal dose provide a general idea of the toxicity of venom. For each venom, by experimentation on animals with known doses of venom, it is possible to determine the minimum lethal dose (MLD) per kilogram of body weight of the animal. For lethal toxicity determination of each batch of venom (cobra and Russell's viper) several groups of mice were taken, subcutaneous injections were given at different doses, which were increased or decreased in 0.01 mg step until 100% mortality was obtained with the highest dose. All deaths of animals during 24 hours following injection were ascribed to venom toxicity.

Before starting of each set of experiments the MLD determination was done on several mice for having a general idea of the toxicity of the venom used in the experiment.

7. Methods of Isolation of Cobra and Russell's Viper Venoms (heated at 100°C for 30 Minutes) by Sephadex Gel Filtration Technique

Details of the methods are described in Chapter V.